Prevalence of glucose-6-phosphate dehydrogenase deficiency in Southern Province, Zambia

by
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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme critical for protecting red blood cells from oxidative damage. G6PD deficiency is an X-linked recessive disorder caused by mutations in the G6PD gene and affects nearly 400 million people worldwide. Among G6PD deficient individuals, it is common to experience premature breakdown of red blood cells in the face of oxidative stress, such as administration of the antimalarial drug primaquine. Primaquine is effective against the late stage gametocyte of *Plasmodium falciparum* and therefore useful in disrupting transmission. In Macha, a town in the Southern Province of Zambia, malaria transmission has declined significantly in the past decade. It is hypothesized that a control strategy, such as single-dose primaquine treatment, may be an effective tool for potential malaria elimination. Unfortunately, there is little information available on local G6PD deficiency prevalence. Thus, the primary aim of this project was to determine the prevalence of G6PD deficiency in Macha. We focused on the G6PD A- genotype, as it is the most prevalent genotype for the deficiency in Africa. In order to determine the prevalence of G6PD deficiency, DNA was purified from 56 blood samples collected from Macha in June 2014. Touchdown polymerase chain reaction (PCR) was done and PCR products were digested with restriction enzyme NlaIII to determine which individuals have the G6PD A- genotype. Deficient individuals presented a mutation from guanine to adenine at the 202nd base pair site. The prevalence of G6PD deficiency in Macha was 8.9%. This information will help inform the Zambian Ministry of Health's potential malaria elimination strategy involving mass drug administration of primaquine.

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Background

Nearly half of the global population is at risk of contracting malaria [1]. Between 2000 and 2015, the rate of new malaria cases decreased by 37% worldwide [1]. This resulted in a 60% reduction in malaria death rates among all age groups, with the exception of a 65% decrease seen in children under five years of age [1]. Although there seems to be a global decrease in malaria infection, areas such as Sub-Saharan Africa continue to carry a disproportionately high global burden of disease. For instance, in 2015, 89% of malaria cases and 91% of malaria deaths were in this region [1]. The World Health Organization (WHO) is attempting to tackle this issue with single-dose primaquine administration. Knowledge of the malaria lifecycle is crucial for understanding the potential consequences of this approach.

Malaria Life Cycle

During a blood meal, a malaria-infected female *Anopheles* mosquito injects sporozoites into the human host [2]. These sporozoites infect liver cells and mature into schizonts, which go on to rupture and release merozoites [2]. It is important to note that in the case of *Plasmodium vivax* and *Plasmodium ovale* infections, there is a dormant liver stage [2]. In the absence of a dormant liver stage, the merozoites are released and begin infecting red blood cells, starting the initial human blood stage [2]. During this stage, some merozoites leave the asexual replication cycle and develop into sexual forms of the parasite called gametocytes [2]. The gametocytes are ingested by the *Anopheles* mosquito during a blood meal and begin the sporogonic cycle [2]. These gametocytes are released into the gut of the mosquito and proceed to mature into gametes [2]. Male and

female gametes fuse to form zygotes and develop into moving ookinetes [2]. The ookinetes burrow into the mosquito midgut wall and later form oocysts [2]. Each oocyst produces thousands of active haploid forms called sporozoites that travel from the body cavity of the mosquito to the salivary gland [2].

Primaquine

The intricacies of the malaria life cycle make it complex and unpredictable. Antimalarial drugs target the malaria life cycle at different stages [3]. Some drugs act against early gametocytes during the blood stage. However, the antimalarial drug primaquine, has potent gametocytocidal activity against mature *Plasmodium falciparum* gametocytes and prevents relapse in cases of *Plasmodium vivax* infection [3]. Primaquine is key to elimination in low transmission areas, as it rapidly reduces the transmission of gametocytes between the human host and vector while containing the spread of artemisinin resistance [3].

However, Sub-Saharan Africa's history of high malaria infection has led to the evolution of protective genetic traits against the disease, such as glucose-6-phosophate dehydrogenase (G6PD) deficiency [4]. This idea was presented in early studies that suggest *P. falciparum* and *P. vivax* parasites prefer to invade younger erythrocytes, which possess high levels of G6PD enzyme [5]. Consequently, the parasites do not prefer to invade older erythrocytes, as enzyme levels are low in these cells [5].

Ruwende et. al conducted a case-control study on over 2,000 African children to test the potential protective effect of G6PD enzyme. They found that children who had the African form of G6PD deficiency (G6PD A-) reduced their risk of

contracting malaria by 46 to 58% [6]. This study concluded that the selective advantage of resistance to malaria was counterbalanced with selective disadvantageous results of G6PD deficiency [6].

The disadvantageous results of G6PD deficiency appear in the face of oxidative stress. For instance, treatment with primaquine can cause varying degrees of hemolysis in patients with G6PD deficiency. The extent of hemolysis is contingent on the dose and duration of primaquine treatment as well as the degree of G6PD deficiency [7].

Some experts in the field argue that a minimal risk is associated with the single-dose primaquine regimen. Reports show that 14 deaths have been reported in six decades of primaquine use in 200 million people [7]. When calculating the prevalence of mortality using studies that report a known denominator, the estimated value is 1 in 621,428 (upper 95% CI: 1 in 407,807) [7]. However, the statistics remain challenged by the counterargument that without a practical point-of-care field test, primaquine treatment decisions may pose risky hemolytic threats to patients with G6PD deficiency.

Enzyme Structure

The glucose-6-phosophate dehydrogenase (G6PD) gene is located on the long arm of the X chromosome at position 28 [4]. The G6PD monomer consists of 515 amino acids and has a molecular weight of approximately 59 kDa [4]. The monomer has two domains - the N-terminal domain and beta+α domain [4]. The N-terminal domain contains a β-α-β nucleotide binding site while the β+α domain consists of an antiparallel nine-stranded sheet [4]. As depicted in Figure 1, the dimer interface lies in a barrel arrangement and contains a conserved peptide region acting as a substrate binding site [4]. Certain

resolutions display a coenzyme NADP+ molecule in every subunit of the tetramer close to the dimer interface [4]. The enzymatic activity is dependent on the stability of the active quaternary structures [4].

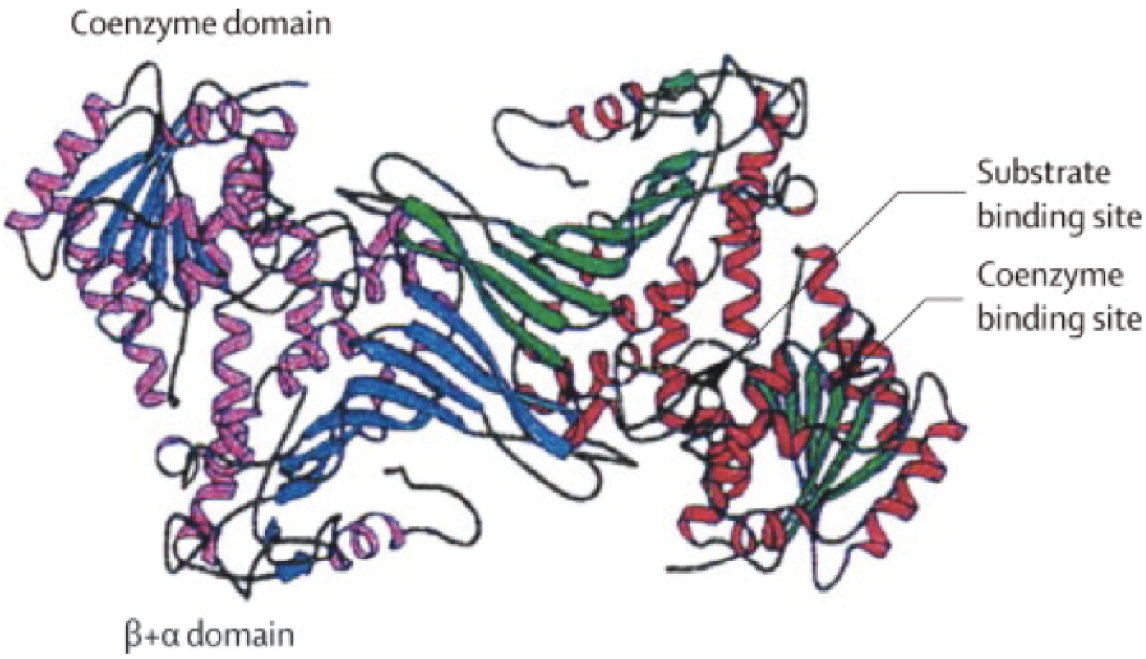

Figure 1. Three-dimensional model of G6PD dimer [4]

Enzyme Function

Glucose-6-phosphate dehydrogenase catalyzes the first reaction in the pentose phosphate pathway, referred to as the committed step [4]. G6PD provides reducing power in the form of NADPH by converting glucose-6-phosphate to 6-phosphogluconolactone [4]. The conversion permits regeneration of the reduced form of glutathione. Glutathione is essential for decreasing the amount of hydrogen peroxide and oxygen radicals in the body.

Glucose-6-phosphate dehydrogenase plays a role in virtually all cell types due to

its involvement with the normal processing of carbohydrates [4]. It has a particularly important role in red blood cells, protecting them from premature destruction and ensuring oxygen transportation throughout the body [4]. The synthesis of NADPH by glucose-6-phosphate is crucial for red blood cells as they lack other NADPH-producing enzymes and have an increased susceptibility to damage from oxidative stress [4].

Enzyme Deficiency

G6PD deficiency is an X-linked recessive disorder that afflicts nearly 400 million people worldwide [4]. As a result of the X-linked inheritance pattern, the heterozygous genotype, BA-, is only present in females. The biological causes of the deficiency range from a reduction in the number of enzyme molecules to structural differences in the enzyme [4]. In most cases, G6PD deficiency is due to enzyme instability resulting from amino acid substitutions [4].

With more than 300 variants, G6PD deficiency is prevalent among individuals from African, Asian, and Mediterranean descent [8]. G6PD Mediterranean and G6PD A- are the two most common variants among humans. G6PD Mediterranean is characterized by a substitution of cytosine to thymine at position 188 (SER188PHE) [9]. This genotype is prevalent in the Middle East [9].

The G6PD A- variant is responsible for the high prevalence of deficiency among African populations [8]. This variant differs from the wild-type G6PD B genotype by two amino acid substitutions, guanine to adenine at positions 376 (VAL68MET) and 202 (ASN68ASP) [8]. The first missense mutation encodes the allelic change from G6PD B variant to G6PD A variant and the second mutation differentiates the G6PD A- genotype

from the G6PD A genotype [8]. The G6PD A genotype has an enzymatic activity of 85% whereas the A- genotype has activity levels around 12% [8].

The G6PD Mediterranean and G6PD A- genotypes represent opposite ends of the severity spectrum for hemolysis associated with primaquine treatment. Adverse reactions to primaquine are profound in the G6PD Mediterranean variant and mild in the G6PD A- variant. Nevertheless, severe hemolytic reactions can still occur among individuals with G6PD A- genotype. Hemolytic risk is difficult to predict given the substantial variability in G6PD activity among individuals with the same genotype, and even within the same individual over time.

Hemolysis

There are a few proposed mechanisms for primaquine-induced hemolysis, although the process is still not understood in its entirety [3]. One hypothesis suggests that the 5-hydroxyprimaquine metabolite is dominated by its oxidized quinoneimine species in G6PD deficient red blood cells [3]. The quinoneimine species reacts with the heme moiety of hemoglobin and cause its displacement to the lipid bilayer of red blood cells [3]. The displacement of the heme moiety results in acute intravascular hemolysis [3]. Freely circulating hemoglobin has the potential to cause the most severe clinical symptoms, such as renal failure [3].

Another mechanistic study suggests that glutathione is oxidized to glutathione disulfide and lost from the red blood cell [10]. Lastly, some hypotheses point to the formation of Heinz bodies as a result of denatured and aggregated hemoglobin on the inner surface of the cell membrane [3].

Malaria and G6PD Deficiency

Work done by Howes et al. estimated the frequency of G6PD deficiency across malaria endemic countries by creating a prediction map model. Representative community surveys of phenotypic G6PD deficiency taken from 1,734 sites were used to generate a Bayesian geostatistical model [3]. This model created a G6PD deficiency allele frequency map across malaria endemic countries. Unlike existing published maps of G6PD deficiency, the maps generated by Howes et al. produced estimates that were weighted according to population size. This ensures the estimates were unbiased as weighted calculations correct for possible biased distributions.

Current published maps pose many limitations. For instance, existing maps summarize average frequency data to national levels masking sub-national variation [3]. Some fail to exclude potentially skewed or unrepresentative survey samples while others disregard the prevalence of the deficiency in females, and most maps do not have a framework to incorporate spatial heterogeneity into population-affected estimates [3].

The results from this study showed that Sub-Saharan Africa had the highest continental-level deficiency frequency with 65.9% of the land area having a prevalence greater than or equal to 5% [3]. Additionally, 37.5% of the area had a median G6PD deficiency prevalence greater than or equal to 10% [3]. Overall, the study calculated a G6PD deficiency prevalence of 15-20% in Zambia and 5-15% in Botswana [3]. Howes et al. concluded that G6PD deficiency is widespread and spatially heterogeneous across most malaria endemic countries.

A study conducted by the WHO Working Group gave separate but similar statistics regarding the global distribution of G6PD deficiency. They noted that

approximately 7.5% of the world population carries one or two genes for G6PD deficiency [11]. Although the deficiency is an X-linked disorder, females contribute to nearly 10% of the deficient population due to the high frequency of the gene and high incidence of consanguineous marriages [11]. Ultimately, the WHO Working Group found a comparable result to Howes et al. ranking parts of Africa with the highest global prevalence of G6PD deficiency at 35%.

Project Aims

The goal of my project was to inform a malaria elimination strategy proposed by the Zambian Ministry of Health (MoH) by determining the prevalence of glucose-6-phosphate dehydrogenase deficiency in Macha, a town in Southern Province. Macha is in the Choma District of Zambia, one of the three project sites for the Southern Africa International Centers of Excellence for Malaria Research (ICEMR) team. The number of malaria cases in Macha has significantly decreased over the last ten years due to the introduction of bed nets and artemisinin combination therapy. However, complete malaria elimination can only be achieved by preventing transmission. Thus, the MoH hopes to administer the antimalarial drug primaquine, to disrupt the current low levels of transmission and eliminate malaria.

Macha, along with the Southern Province, is populated with individuals from the Tonga tribe. Similar to many Zambian tribes, the Tonga practice interbreeding. Consequently, the genetic makeup of members from one tribe is generally assumed to be homogenous. Under this assumption, the results achieved from our project in Macha may be relevant to Southern Province.

I hypothesized that the prevalence of G6PD deficiency in Southern Province was 10-20% - a range that fell in between results from previous studies. Although the deficiency is common among certain ethnic groups, there is limited information on the distribution of G6PD deficiency in Zambia. By determining deficiency levels, I hope to provide the MoH valuable insights to bring about a positive regional impact.

Methods

Study Site

The ICEMR study sites are comprised of three regions with distinct malaria transmission and control [12]. As shown in Figure 2, the three sites are Choma and Nchelenge Districts in Zambia and Mutasa District in Zimbabwe. Choma District has successfully accomplished malaria control while Nchelenge District has not effectively controlled malaria transmission [12]. The ineffective control in Nchelenge is apparent by the high number of malaria cases each year. Unlike Choma and Nchelenge, Mutasa District formerly achieved successful malaria control but is now in a period of resurgence [12].

The focus of this project is on the Choma District in Southern Province, Zambia. In 2011, the population of Choma stood at 204,989 [12]. This drought-prone region receives the lowest mean annual rainfall in the country and has an unpredictable rainy season between November and April [12]. The primary vector in Southern Province, *Anopheles arabiensis*, peaks during the rainy season [12].

Fortunately, the Southern Province is one of the few regions in Zambia with low parasite prevalence [12]. From 2006 to 2010, the prevalence of malaria parasitemia in

children under 5 dropped from 13.7% to 5.7% [12]. The decline has been attributed to the use of effective artemisinin combination therapy in conjunction with the scale up of insecticide treated bed nets [13].

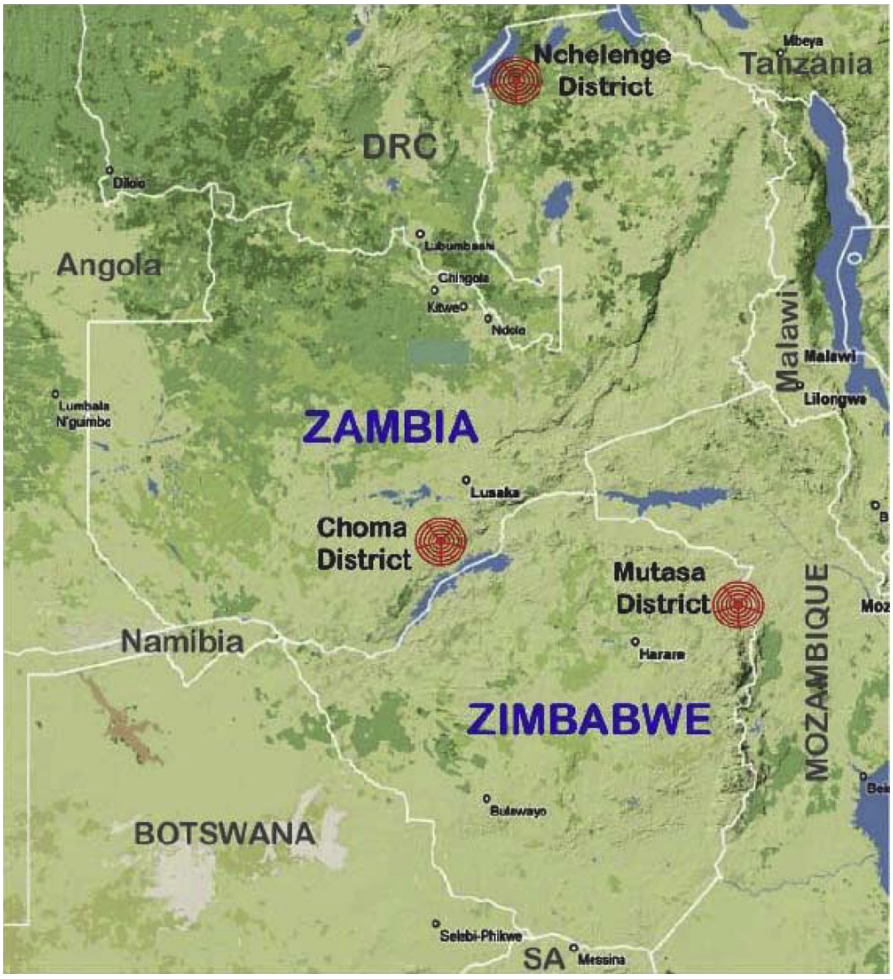

Figure 2. Three Southern Africa ICEMR sites – Choma, Nchelenge, and Mutasa Districts [12]

Sample Selection

The 56 whole blood samples used for my graduate thesis analysis were collected from the Choma District in June 2014 as part of an active screening project conducted by ICEMR. The active case detection participants were enrolled in prospective longitudinal

and cross-sectional surveys of malaria parasitemia in the catchment area of Macha Hospital in Southern Province, Zambia [13]. Satellite images were constructed using Quickbird to form a sampling frame to randomly select households (Figure 3) [13]. However, with low levels of malaria transmission in Macha, the team was unable to capture a substantial number of cases through active case detection. Thus, ICEMR created the Step-D project to replace active screening.

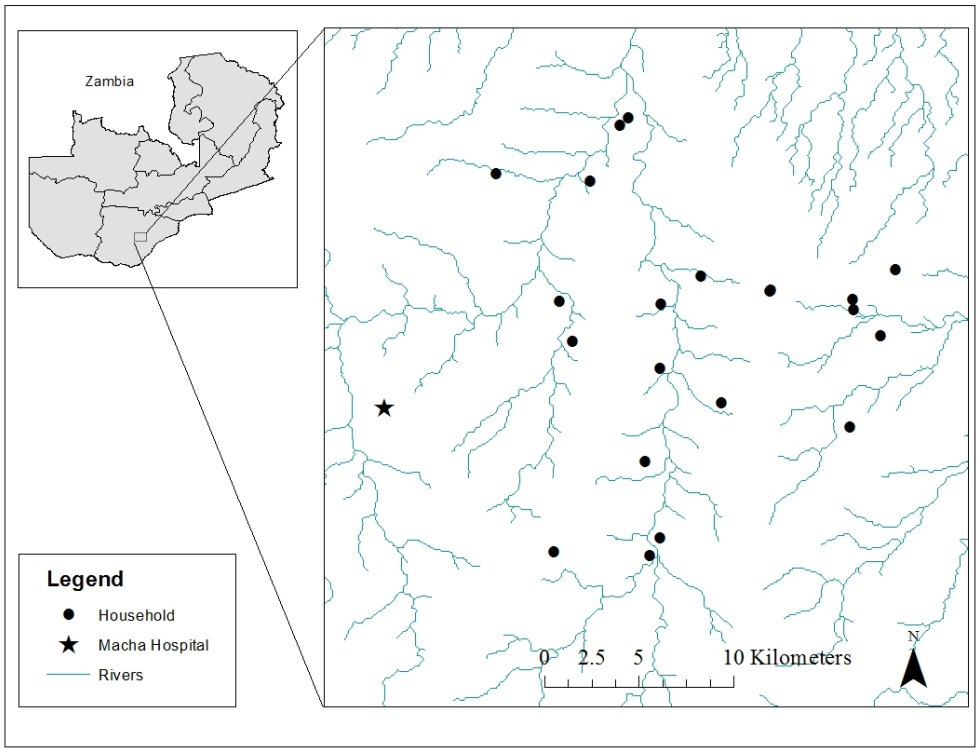

Figure 3. Map used to randomly select households for active case detection in Macha [13]

Step-D and Enhanced Step-D Projects

The MoH instituted the step-D program to assist with malaria surveillance and elimination. The goal of the step-D program was to detect malaria index cases in the Choma district with the help of local clinic nurses and staff members. The recruited

facilities were provided cell phones and asked to Short Message System (SMS) the Macha Research Trust (MRT) when a patient was diagnosed with malaria. After receiving notice about a malaria case from a health clinic, the MRT field team would determine the location of the index case household and create maps to locate all houses within a 140-meter radius from the index case household. The homes within range were eligible to participate in the program.

To assess the adequacy of the 140-meter radius, ICEMR expanded to a 250-meter radius around the index case household. The expansion led to the formation of the enhanced step-D program. In July 2015, I collected 150 samples from participants involved with enhanced step-D. Those samples will be used to conduct a cross sectional analyses at a later date.

Location of Houses

In order to determine the exact location of the index case household and the houses within a 250-meter radius, the field team created Geographical Information Systems (GIS) maps. An example of a GIS map used by the MRT field team is shown below in Figure 4.

After determining the coordinates of each house, a member from MRT would visit the families and provide a light trap to use during the night. During this visit, the households were informed about the enhanced step-D program and the role of the light trap in informing the study about parasite diversity. The enrolled participants were asked to use the light traps until the next day when the field team returned for sample collection.

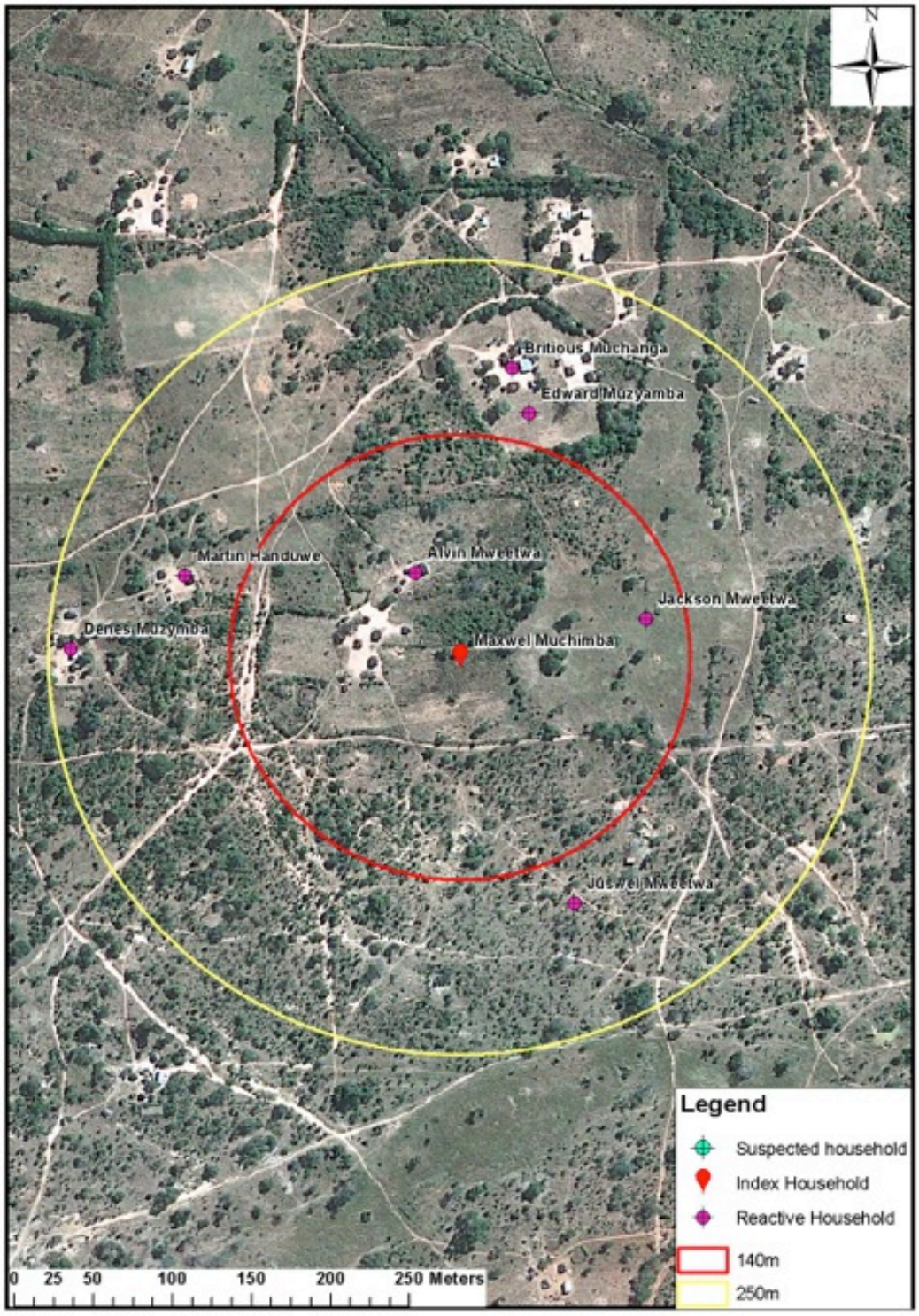

Figure 4. GIS map used for enhanced step-D project

Enhanced Step-D Fieldwork

The next day, the field team set up stations central to multiple houses and began the sample collection process (Figure 5). One person from the MRT field team was responsible for administering a survey while two MRT members were in charge of taking blood samples. The survey consisted of questions that assess the use of bed nets, antimalarial access and intake, previous malaria infections, and presence or absence of clinical malaria symptoms. The blood was obtained through a finger prick and placed in capillary tubes as well as on filter paper (Figure 6). The blood sample was also used for a rapid diagnostic test (RDT). Individuals who tested positive for malaria by the RDT were treated on site with artemisinin combination therapy (Figure 7). Additionally, the field team conducted a test to check the participants' hemoglobin levels.

Figure 5. Central field station set-up

Figure 6. Finger prick and capillary tube collection method in the field

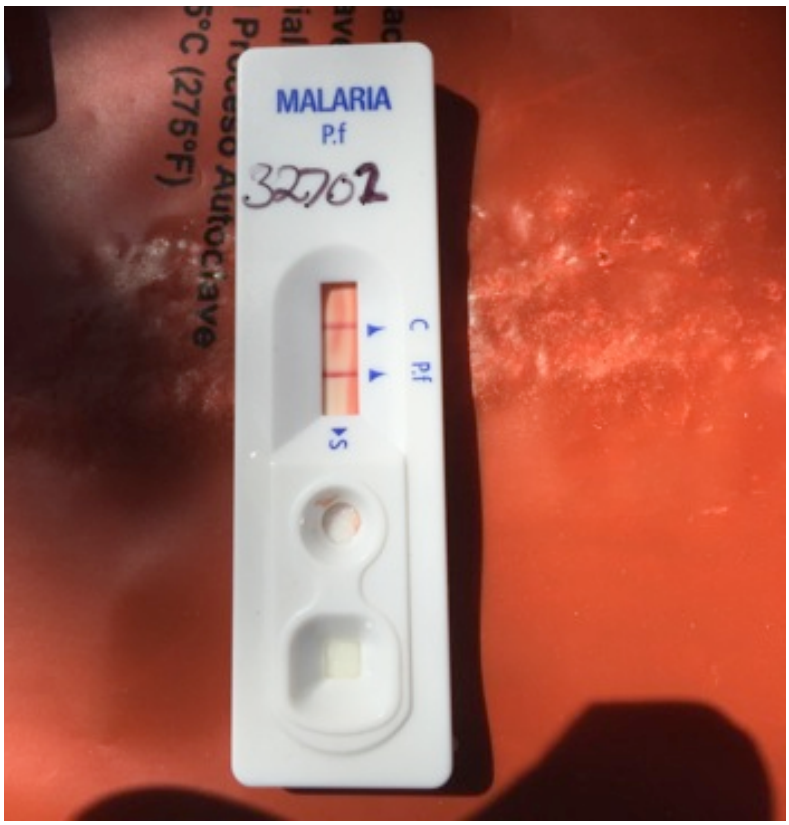

Figure 7. Positive RDT detecting of *P. falciparum* infection

Purification

The protocol from the Qiagen QIAmp DNA blood mini kit was used to purify the red blood cell fractions (Figure 8). The cells were lysed to release the DNA, which was bound to the spin column then washed to remove residual contaminants. The final elution contained purified DNA that was used for the experiment. A complete explanation of the protocol can be found in the Appendix.

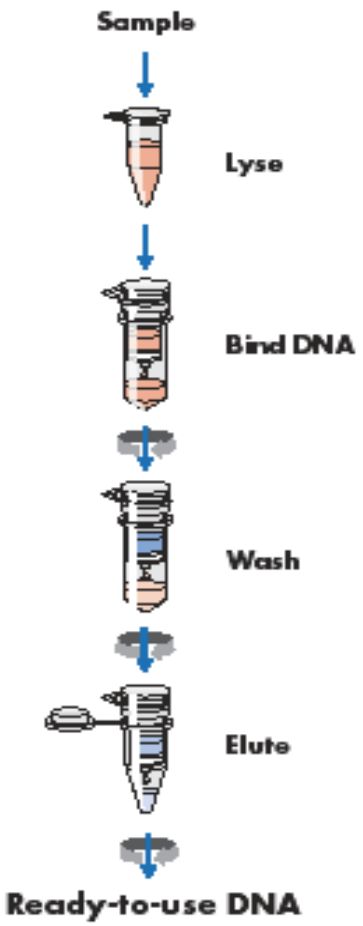

Figure 8. Methods for QIAamp DNA blood mini kit [14]

Polymerase Chain Reaction

A touchdown polymerase chain reaction (PCR) was conducted to amplify the region containing the G6PD gene. In a touchdown PCR, the initial annealing temperature is higher than the optimal melting temperature of the primers and is gradually reduced over subsequent cycles.

The methods section presented in the Fanello et al. paper was initially followed to perform the PCR. However, when using the study's protocol, no signals appeared in the gel images. This led to three months of troubleshooting. Multiple troubleshooting

strategies were attempted, such as making new dNTP, diluting and concentrating the DNA by 10x, and changing the gel concentration (Figure 9). A strong signal was finally observed when red blood cell fractions were used in place of the dry blood spots and a Phusion High Fidelity DNA-Polymerase replaced the Taq polymerase. A stronger signal was achieved due to larger sample quantity and stronger binding of the polymerase. The Phusion High Fidelity polymerase was less error-prone than the Taq polymerase allowing for more precise binding to the DNA in our samples.

A) **B)** **C)**

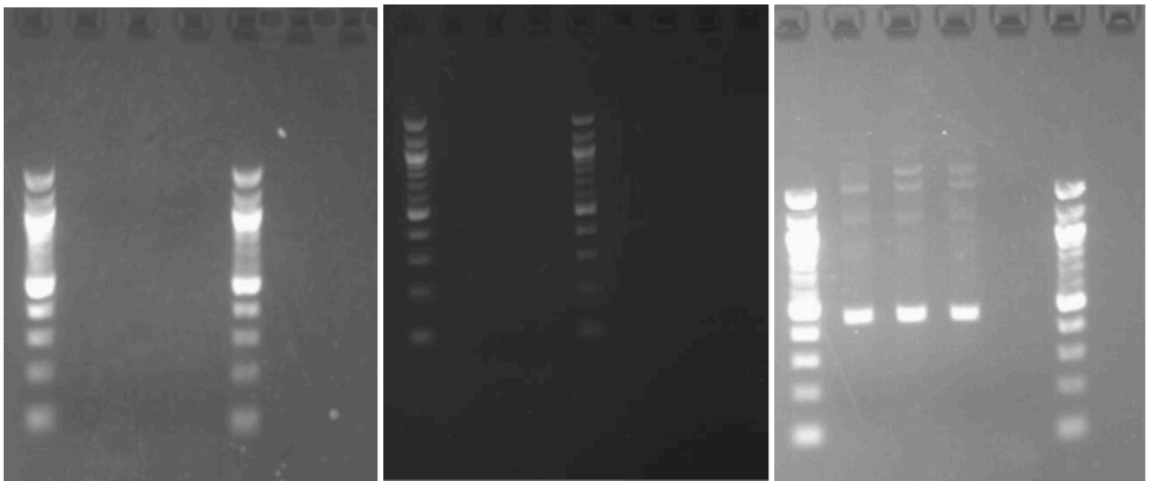

Figure 9. Troubleshooting results a) new dNTP b) DNA dilution 10x c) Phusion polymerase indicating band at 461 bp

Once the samples were purified using the QIAamp kit, a master mix was made using 5µL of high fidelity buffer, 2µL of 5µM dNTP, 1.25µL of forward and reverse primers, 0.75µL of DMSO, 0.25µL of Phusion polymerase, and 11.5µL of purified H_2O. The amounts listed are for one sample. The master mix was then vortexed, centrifuged,

and added to centrifuge tubes. 22µL of master mix was added to 3µL of sample template. This tubes was centrifuged at 4° C then placed in the PCR machine at the conditions listed in Table 1.

Table 1. PCR conditions

Initial Denaturation	98° C	30 seconds
25-35 Cycles	98° C	5-10 seconds
	45-69° C	10-30 seconds
	72° C	15-30 seconds
Final Extension	72° C	5-10 minutes
Hold	4° C	∞

Gel and Imaging

In order to visualize the PCR product, a 2% agarose gel was made using 1.0 gram of agarose gel powder and 50ml of 1x TAE buffer. This mixture was weighed then microwaved until all of the powder was dissolved. To compensate for evaporated liquid, the appropriate amount of water was added to achieve the initial weight. Finally, 2.5µL of ethidium bromide was added to the liquid gel, which was then mixed and poured into the set tray to solidify.

To load the gel, 1µL of loading dye, 5µL of sample, and 1.4µL of 1,000kb DNA ladder were used. The gel was run at 100 watts on the DC setting for 25 minutes. Once the gel was finished running, it was imaged to confirm proper amplification, indicated by a band at 461bp.

Restriction Enzyme Digest

The next step was to conduct an NlaIII restriction enzyme digest on the PCR product. This digest was performed to observe the appropriate cuts for G6PD A- genotype. The NlaIII enzyme makes a cut at the 202nd base pair site in G6PD deficient individuals indicating a substitution from guanine to adenine.

Similar to the PCR, a master mix was made for the restriction enzyme digest. The following mixture is for one sample: 3µL of 10x NlaIII buffer, 0.6µL of NlaIII enzyme, and 6.4µL of purified H_2O. The master mix was vortexed and centrifuged. 10µL of sample was added to 20µL of the PCR product and placed into the PCR machine. The digest conditions are listed in Table 2.

Table 2: NlaIII digest conditions

37° C	60 minutes
65° C	20 minutes
4° C	∞

The same procedure was used for gel preparation, loading, and analysis as stated above.

Results

Demographics

A metadata spreadsheet was used to retrieve information on each of the study participants. The metadata provided date of birth, age, gender, date of sample collection, country, district location, GPS coordinates, health status, and parasite detection/load (Table 1). The 56 samples analyzed in this study were made up of 54% males (n=30) and

46% females (n=26). The median age among men was 18 years and the interquartile range was 36.8 years. Similarly, the median age among women was 22 years and the interquartile range was 35.5 years.

The 32-year-old male who tested positive for G6PD deficiency was the only individual who did not fall within the normal hemoglobin rage for his age. Normal hemoglobin range for adult males is between 14-18 g/dL [15] [16].

Table 3: Demographics for G6PD deficient individuals in Choma District

Age	Gender	GPS (Longitude, Latitude)	Hemoglobin Level (g/dL)
5	Male	-16.3859419, 26.88840474	13.5
9	Male	-16.26570167, 26.96554556	12.1
9	Male	-16.3859419, 26.88840474	13.1
14	Male	-16.30870098, 26.97903177	13.3
32	Male	-16.3859419, 26.88840474	12.5

Expected Results

Figure 10 shows the expected results associated with each genotype, bearing in mind the heterozygous form is only possible in females. Distinct cuts and band sizes distinguished the respective genotypes. For instance, a sample that was not digested

would present a band at 461bp. This is the same band length that was observed from the PCR if proper amplification was achieved. An individual with G6PD B genotype would show band lengths of 296bp and 165bp. These bands indicate the wild type genotype and normal enzymatic activity. An A- genotype expression would have bands at 173bp and 123bp. And not surprisingly, a heterozygous woman with a BA- genotype would present a combination of bands from the B and A- genotypes. She would display bands at 296bp, 173bp, and 123bp.

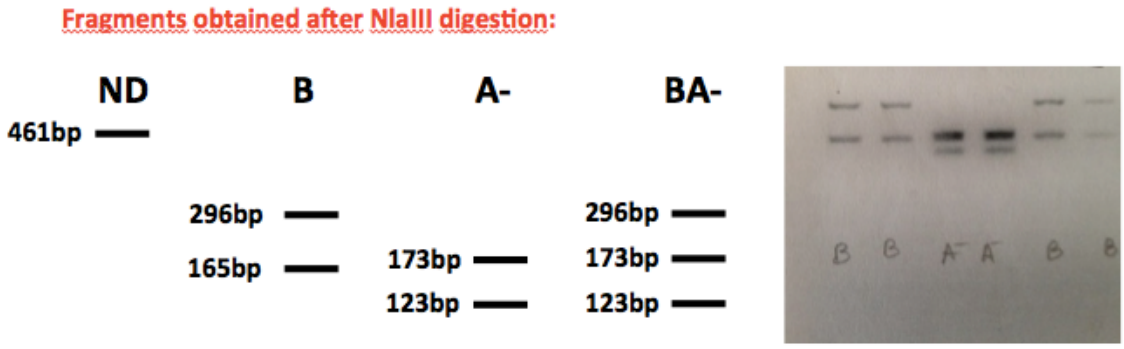

Figure 10. Expected restriction enzyme digest results [8]

Results

Ultimately, we determined a G6PD deficiency prevalence of 8.9% in Choma. Five out of the 56 samples from June 2014 showed positive digest results for G6PD A- genotype (Figures 11 & 12). We were 95% confident that the true value of G6PD deficiency in Macha was between 1.5 and 16.4%.

The 5 individuals who presented bands for the A- genotype were all male participants (Figure 13). Household clustering was evident among deficient individuals. Three of the individuals who were positive for G6PD deficiency lived in the same

household; their ages were 5, 9, and 32 years. The two other participants, aged 9 and 14 years, represented different households in the Choma District.

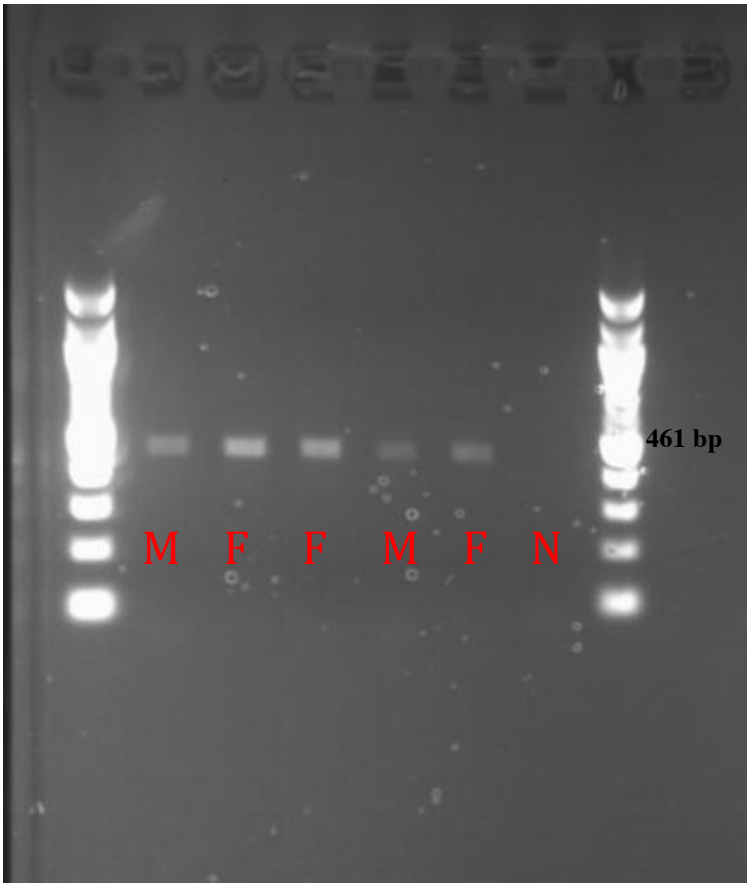

Figure 11. PCR product results at 461bp (m: male, f: female, n: no template control)

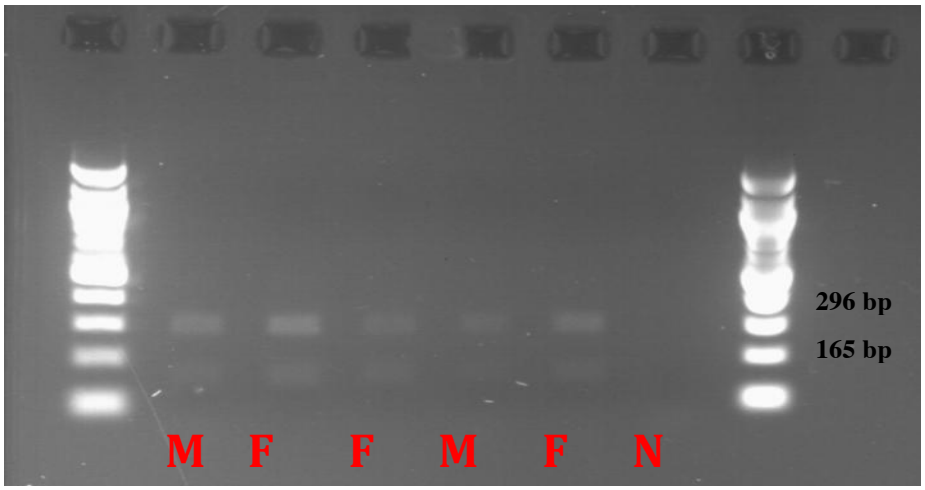

Figure 12. Digest results indicating G6PD B genotype for all samples

A) **B)**

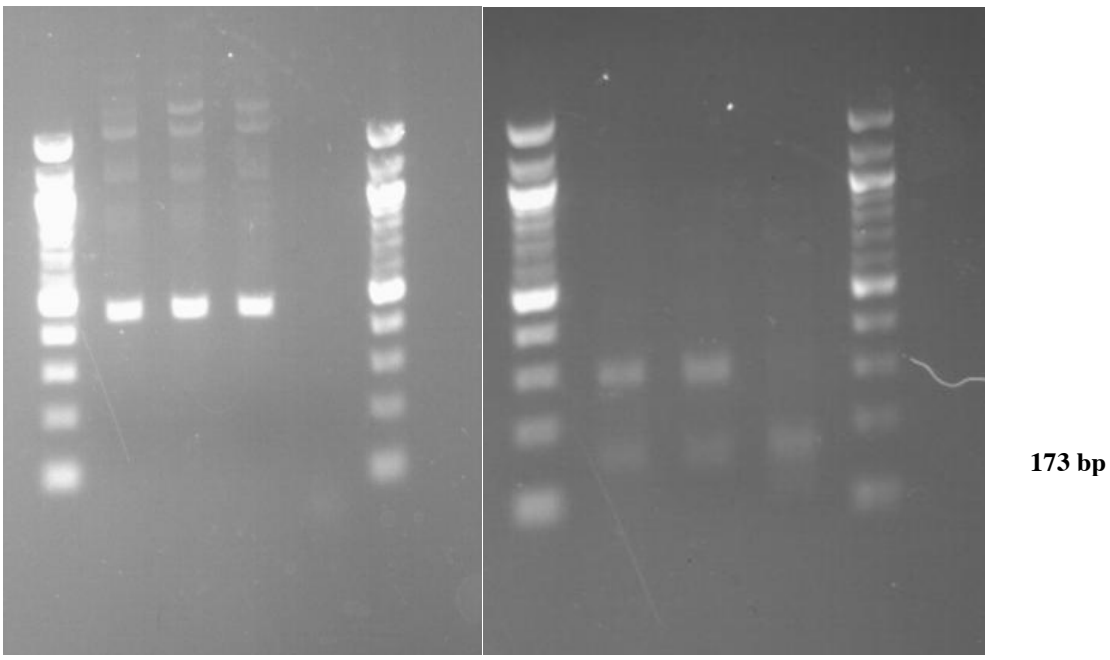

Figure 13. a) PCR product for 3 male samples b) digest results showing G6PD A-genotype for third sample

Discussion

Conclusions

This study aimed to determine the prevalence of G6PD deficiency in Macha, Zambia, a town in Southern Province. After purifying the samples, performing PCR, and conducting a restriction enzyme digest, we concluded a prevalence of 8.9% for the G6PD A- genotype. The 95% confidence interval was 8.9% +/- 7.5%.

Limitations

As mentioned previously, the small sample size of this project may affect the generalizability of the obtained results. However, we can assume a similar genetic make up for most individuals living in Southern Province, as they are part of the Tonga tribe. Historically, the Tonga tribe has maintained a reputation for interbreeding allowing us to make this assumption.

Another limitation may be the appearance of signal in the negative control on the PCR gel image. Signal in the water control is a sign of potential contamination. We faced this problem towards the end of our project. However, we confirmed that this issue did not affect our results; the bands produced from the samples showed a much stronger signal and were eventually digested unlike the band in the negative control.

Additionally, the use of water as the negative control may not suffice as the sole control. Thus, it would be beneficial to incorporate the use of a target gene apart from the G6PD gene to confirm that we are amplifying the correct gene region. It would also be useful to include a positive control, such as a sample that has been amplified previously. The positive control plays an important role in the event bands are not seen in the gel

image.

Future Directions

Due to the small sample size used for this study, I will analyze the 150 samples from July 2015 in the upcoming months. This will hopefully increase the validity of our results. I am hoping to finish the 2015 samples by April in an effort to publish my results.

In future studies, recruitment should be done using individuals from other districts within Southern Province. Moving outside of the Choma District will provide a more diverse sample population, thereby increasing generalizability of the results.

In addition, conducting future analyses will inform existing literature on G6PD deficiency. There are theories in the field about possible protective effects of G6PD deficiency against malaria [4]. Additional work could potentially uncover a possible correlation between high malaria transmission and high prevalence of G6PD deficiency.

Because G6PD deficiency can result from mutations at multiple base pair sites, it is important for future studies to perform a biochemical analysis in addition to the PCR and restriction enzyme digest. This provides an assessment of full enzymatic activity rather than limiting the study to the 202nd base pair site. Fortunately our methods sufficed for this project because the study population was mainly at risk for the A- genotype.

Significance in the Field

There is limited published literature on G6PD deficiency in Sub-Saharan Africa. In fact, many studies fall short of reliable methods to determine deficiency levels. As

mentioned previously, predictive map models leave out critical information or include skewed data when producing estimates. In addition, it is very difficult to distinguish heterozygous deficient patients from healthy individuals without reliable methods for diagnosis. Because our methods involved laboratory techniques, we can achieve conclusive results and detect changes at a genomic level. I hope the results from my graduate thesis project will play a significant role in informing malaria control policy and other large-scale initiatives taking place in Sub-Saharan Africa.

The National Malaria Control in Zambia has been informed of my results and is in the process of reviewing their primaquine administration strategy. A final decision has not yet been reached but will hopefully be made within the next year. Without a strategy to complement the current malaria control techniques in Macha, the area is at risk for a resurgence of high malaria infection. Thus, the MoH needs to take action now by creating a safe and practical method to eliminate malaria while transmission is low.

My personal recommendation to the Zambian MoH is to assess the ethical dilemma at hand before implementing policy-level changes. One suggestion that may aid in the decision-making process is conducting a population screening of newborn babies for G6PD deficiency. This would be particularly important among males but also in areas with a high or unknown prevalence. Given the complexity of both malaria and G6PD deficiency, there may not be one single solution that will ensure safe and effective primaquine treatment. Based off the data from this project, I am inclined to support single-dose primaquine administration in Macha. I hope experts can continue making progress on the malaria vaccine and RDTs for G6PD deficiency to help the Zambian MoH create a multi-pronged solution in a timely manner.

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Appendix

Purification protocol

Red blood cell fractions were purified using a Qiagen QIAamp DNA blood mini kit (Figure 8). To begin the process, the blood samples were brought to room temperature and the hot water bath was heated to 56° C. The protease and buffers were prepped with ethanol and the buffers were brought to room temperature. The following procedure was employed for the purification process:

1. Pipet 20µL of Qiagen protease into a 1.5ml tube.
2. Add 200µL of sample to this tube. 130µL of PBS was added to each sample in order to attain a total volume of 200µL.
3. Add 200µL of buffer AL to the sample then pulse vortex for 15 seconds.
4. Incubate samples at 56° C for 10 minutes.
5. Centrifuge the 1.5ml tube briefly and add 200µL of ethanol (96-100%) to sample.
6. Pulse vortex for 15 seconds then centrifuge briefly. This ensures optimal binding of the DNA to the QIAamp membrane before the sample is loaded into a spin column.
7. Apply the mixture from above to a mini spin column without wetting the rim. Close the cap and centrifuge at 8,000 rpm for 1 minute. During this step, DNA is adsorbed onto the QIAamp silica membrane.
8. Place the spin column in a clean 2ml collection tube and discard the tube containing the filtrate.
9. Open the spin column and add 500µL buffer AW1. Centrifuge at 8,000 rpm for 1 minute. This is the first of two wash steps that allows complete removal of residual contaminants without affecting DNA binding.

10. Place the spin column in a clean 2ml collection tube and discard the tube containing the filtrate.
11. Add 500 µL Buffer AW2 to the spin column without wetting the rim and centrifuge at full speed (14,000 rpm) for 3 minutes.
12. Discard the tube containing filtrate and place in new spin column. Centrifuge at 14,000 rpm for 1 minute.
13. Place the spin column in a clean 1.5ml microtube and discard the tube containing the filtrate. Open the column and add 200µL of buffer AE. Incubate at room temperature for 1 minute then centrifuge at 8,000 rpm for 1 minute.
14. Collect the filtrate in a 1.5ml microtube and discard the spin column.

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Education

2014 – present **JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH**
Department of Molecular Microbiology and Immunology, ScM

2010 - 2014 **EMORY UNIVERSITY**
Alpha Epsilon Upsilon Honor Society, Major: Anthropology and Human Biology (Pre-Med), BSc

Experience

Nov 2014 - present **GRADUATE LABORATORY SCIENTIST**
John's Hopkins Bloomberg School of Public Health | Dr. William Moss

- Working with Ministry of Health to inform potential malaria elimination strategy involving mass drug administration of primaquine in Southern Province of Zambia
- Detecting prevalence of glucose-6-phosphate dehydrogenase deficiency in Macha, Zambia through polymerase chain reactions, restriction enzyme digests, and biochemical analyses
- During Summer 2015, conducted six weeks of fieldwork and collected blood samples for project in Macha

Aug 2014 - present **GRADUATE RESEARCH ASSISTANT**
John's Hopkins Bloomberg School of Public Health | Dr. Stefan Baral

- Provide support for an implementation science study of community and peer-based interventions to address HIV risk among key populations in West and South Africa
- Perform protocol development, analysis of quantitative and qualitative data, survey coding, writing and editing reports, and conducting systematic reviews
- Working knowledge of EpiData and Stata14

May 2012 - Aug 2013 **SUMMER UNDERGRADUATE RESEARCH EXPERIENCE (SURE)**
Emory University | Dr. Thomas Ziegler

- Conducted pilot study for double-blind clinical trial to detect presence of active tuberculosis infection in patients and household contacts by performing metabolomics analysis on plasma samples
- Results showed active infection detected by D-series resolvins and putative *M. tuberculosis* cell wall metabolites

Aug 2011 - May 2012 **CHEMISTRY RESEARCH SCHOLAR**
Oxford College of Emory University | Dr. Lloyd Parker

- Conducted a project on "the effect of metal concentration on absorbance involving the chelation of zinc with ammonium pyrrolidine ditihocarbamate" using ultra-violet spectroscopy
- Further research will examine different metals and ligands

Aug 2011 - May 2012 **PSYCHOLOGY RESEARCH SCHOLAR**
Oxford College of Emory University | Dr. Kenneth Carter

- Established a general survey professors can use to determine if their activities, demonstrations, or assignments are helpful to students
- Collected data from Teaching of Psychology journals 2000-2010

Summer 2011 **PSYCHOLOGY RESEARCH ASSISTANT**
University of College London | CIPA Study Abroad

- Conducted Digit Span and verbal/non-verbal Stroop tasks on primary school children to determine effects of age on executive functioning in relation to cognitive and behavioral inhibition
- Compiled and analyzed results with Statistical Package for the Social Sciences (SPSS)

Leadership

Sep 2015 - present **GRADUATE TEACHING ASSISTANT**
John's Hopkins Bloomberg School of Public Health | Public Health Perspectives on Research

- Introduce students to the substantive and methodological basis for public health research, emphasizing the critical roles of quantitative, qualitative, social, and biological sciences
- Responsibilities include grading assignments, engaging students in the learning process, and attending sessions to answer questions

Oct 2013 - May 2014 **CO-FOUNDER**

Project Threads

- Created service organization that engages Emory students in providing meals and clothing to people experiencing homelessness in Atlanta
- Organization makes weekly inner-city deliveries consisting of catered food from local restaurants and packages of bedding and warm clothes

Sep 2013 - May 2014 **ALTO I**

Ahana A Cappella

- Sing for Emory's sole multi-cultural R&B and hip-hop a cappella group
- Perform weekly at city and campus-wide events aimed at increasing diversity and cultural awareness

Jan 2013 - May 2014 **TUTOR AT MONTCLAIR ELEMENTARY AND SEQUOYAH MIDDLE SCHOOL**

Emory Reads and Volunteer Emory

- Tutor at Title I schools each week and create activities to enhance students' reading and math skills
- Work with teachers to make lessons more interactive to increase retention

Jan 2013 - May 2014 **EXECUTIVE BOARD - JHALAK CHAIR**

Pakistani Student Association

- In charge of writing the script and directing annual PSA play Jhalak, to raise awareness for various political and socioeconomic issues in Pakistan
- All proceeds from the play go to Zindagi Trust, a nonprofit organization that works to improve the quality and accessibility of education to Pakistani children
- 2014 leadership board funded Primary and Higher Education for 2 Pakistani orphans

Aug 2011 - May 2012 **RESIDENT ADVISOR**

Oxford College of Emory University | Jolley Residential Center

- Collaborated with Residence Life Coordinator to plan monthly programs focused on learning and community building
- Responsible for maintaining a healthy environment for the academic and personal growth of residents

Aug 2011 - May 2012 **MEDICAL COLUMN AUTHOR**

Spokesman Newspaper

- Authored articles expounding on medical topics frequently misunderstood by Emory students
- Topics included health repercussions associated with lack of sleep and other problems endemic to college campuses

Jan 2011 - May 2012 **SUPPLEMENTAL INSTRUCTOR & TUTOR**

Oxford College of Emory University | Chemistry 141/142

- Responsible for conducting weekly sessions to review course material
- Frequently met with professor to review lesson plans and address class issues

Publications and Presentations

Future of Malaria Research Conference, 2015, "Determining prevalence of glucose-6-phosphate dehydrogenase deficiency in Macha, Zambia"

PLOS ONE Journal, 2014, "Plasma Metabolomics in Human Pulmonary Tuberculosis Disease: A Pilot Study"

FASEB Journal, 2014, "Plasma High-Resolution Metabolomic Profiling Reveals Upregulation of Specific Resolvins with Pulmonary Tuberculosis"

44th Union Conference on Lung Health Journal, 2013, "Plasma Metabolic Profiling in Pulmonary Tuberculosis"

SURE Journal, 2012, "Plasma Metabolomics Analysis from Patients with Tuberculosis in the Republic of Georgia"

American Psychological Association, 2012, "Classroom Activity Learning Scale"

Emory SURE, 2012, "Plasma Metabolomics Analysis from Patients with Tuberculosis in the Republic of Georgia"

Oxford INSPIRE, 2012, "UV Visible Absorption of Dithiocarbamate Transition Metal Complexes"

Oxford Scholars, 2012, "UV Visible Absorption of Dithiocarbamate Transition Metal Complexes"

Awards and Activities

Emory 100 Senior Honorary, Inductee

President Wagner's Keynote Speaker Committee, Student Liason

Cross Roads 3, Leadership Retreat Participant

Miss Oxford of Emory 2012, 1st Prize